Supporting Information

Europium chelate-labeled lateral flow assay for rapid and multiple detection of β-lactam antibiotics by the penicillin-binding protein

Xiaogang Li [a, #], Zhifeng Pan[b, #], Manyu Li [c], Xinmiao Jia [a], Suhan Zhang [c], Hang Lin [c], Juntao Liu [c], Liangkun Ma [c*]

a Medical Science Research Center, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, 100730

b School of Life Science, Fudan University, Shanghai 200433

c Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, 100730

*Address correspondence to maliangkun@pumch.cn

[#] The two authors contributed equally to this work.

Table of contents

S1 Expression and purification of recombinant proteins

The pET-28a (+)-PBP plasmid was synthesized by Wuxi Qinglan Biotech. Inc(wuxi, Jiangsu, China). *Escherichia coli* BL21(DE3) was transformed with the pET-28a (+)-PBP plasmid. For the lab-scale production of recombinant proteins, a modified protocol for expression was used. Briefly, precultures were grown at 37 ºC for 6 h in 40 ml Luria-Bertani (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl per liter) with 200 rpm shaking to an OD600 of 0.8. The expression of PBP was induced by the addition of IPTG to a final concentration of 1 mM, and the induced cultures were shaken for 16 h at 25 ºC. Cells were harvested and resuspended in 50 mM sodium phosphate pH 8, 500 mM NaCl, and 10 mM imidazole. Cell lysis was performed by a 45-min incubation with 0.25 mg/ml lysozyme at room temperature (RT) followed by 3 freeze-thaw cycles at -196 ºC and 37 ºC, respectively. DNA was digested with 5 μg/ml DNase I for 15 min at RT. Cellular debris was pelleted at 20,000 × g and 4 ºC. The supernatant containing 6x-histidine-tagged recombinant fusion proteins was applied to a His-tag Protein Purification Kit following the manufacturer's instructions (Beyotime Biotechnology).

The purified PBP (as assessed by 10% SDS-PAGE with Coomassie Brilliant Blue staining) was stored at -20 ºC in the presence of 10% glycerol. The protein concentration was determined by the Bradford method.

Amino Acid Sequence of PBP:

MGSSHHHHHHSSGLVPRGSHMRLTELREDIDAILEDPALEGAVSGVVVVDTA

TGEELYSRDGGEQLLPASNMKLFTAAAALEVLGADHSFGTEVAAESAPGRRG

EVQDLYLVGRGDPTLSAEDLDAMAAEVAASGVRTVRGDLYADDTWFDSER

LVDDWWPEDEPYAYSAQISALTVAHGERFDTGVTEVSVTPAAEGEPADVDL

GAAEGYAELDNRAVTGAAGSANTLVIDRPVGTNTIAVTGSLPADAAPVTALR

TVDEPAALAGHLFEEALESNGVTVKGDVGLGGVPADWQDAEVLADHTSAEL

SEILVPFMKFSNNGHAEMLVKSIGQETAGAGTWDAGLVGVEEALSGLGVDT

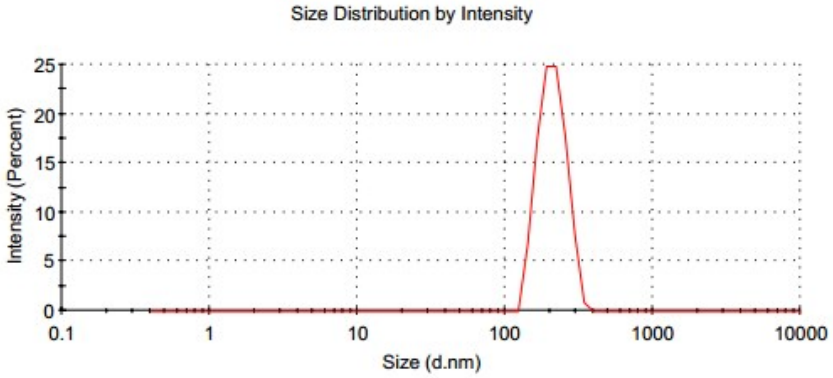
AGLVLNDGSGLSRGNLVTADTVVDLLGQAGSAPWAQTWSASLPVAGESDPF

VGGTLANRMRGTAAEGVVEAKTGTMSGVSALSGYVPGPEGELAFSIVNNGH

SGPAPLAVQDAIAVRLAEYAGHQAPE

Figure S1 Size distribution of pure microspheres (A) and lanthanide chelate-loaded microspheres (B)

(A)



(B)

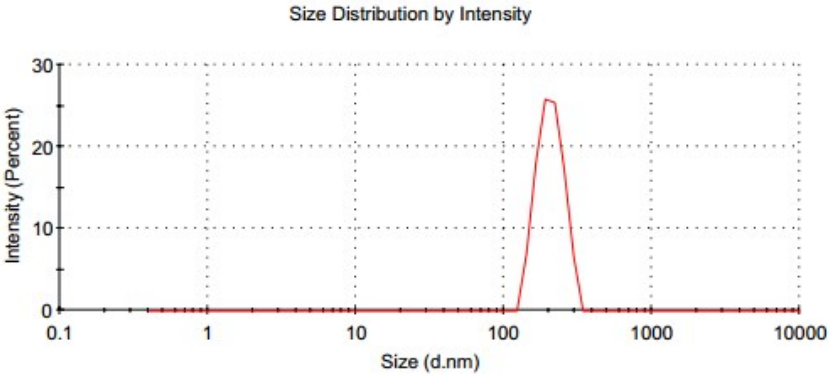
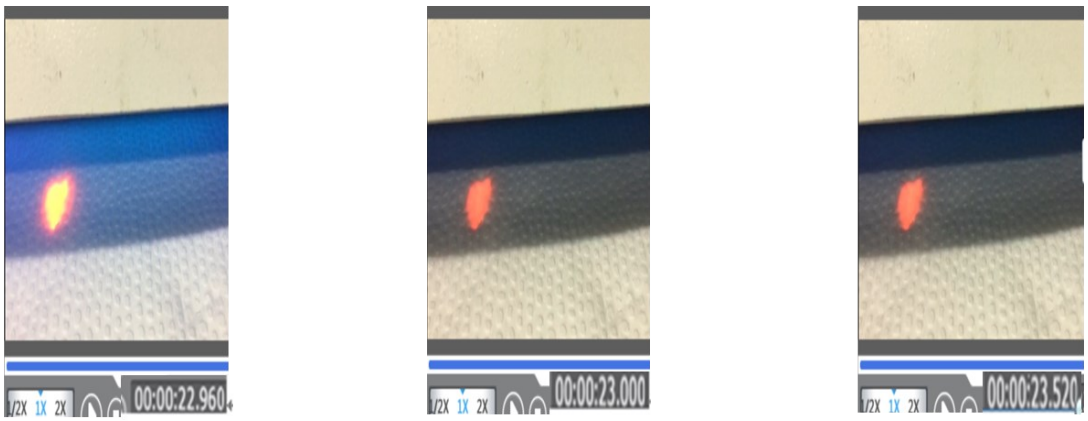


Figure S2 The fluorescence lifetime of europium chelate-loaded polystyrene microspheres

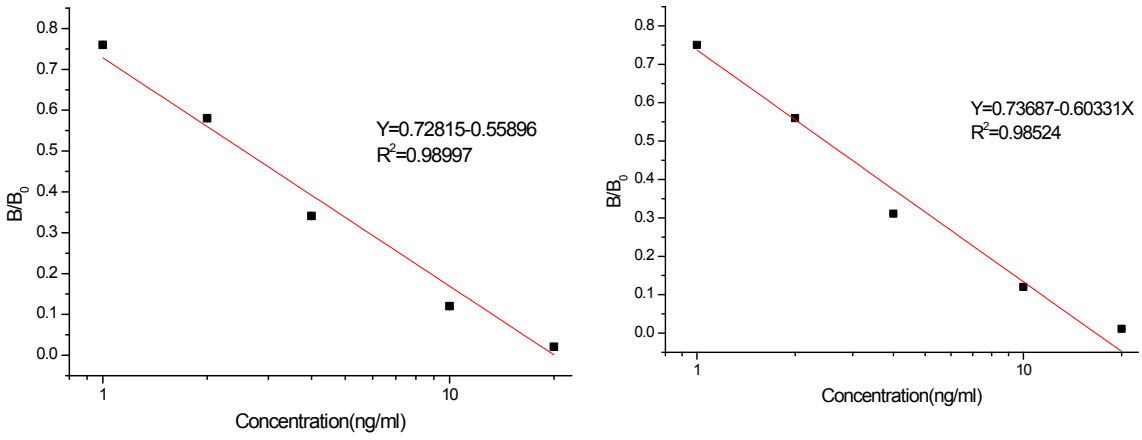


t=0 ms **t=32 ms** **t=500 ms**

Figure S3 Analytical performance of the assay for detecting β-lactams

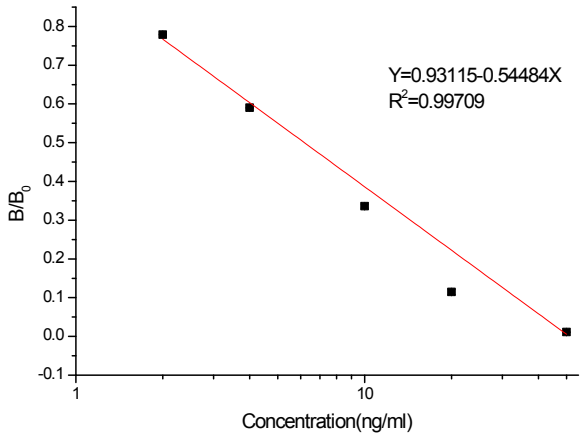
The assay can quantitatively detect β-lactams by measuring the fluorescence signal. To investigate the quantitative determination potential of the detection strategy for β-lactams, penicillin G, amoxicillin, and cefmetazole were employed as positive controls. The standard curve of the assays is shown in Figure S3, indicating the quantitative determination potential of a specific β-lactam antibiotic.

(A) penicillin G

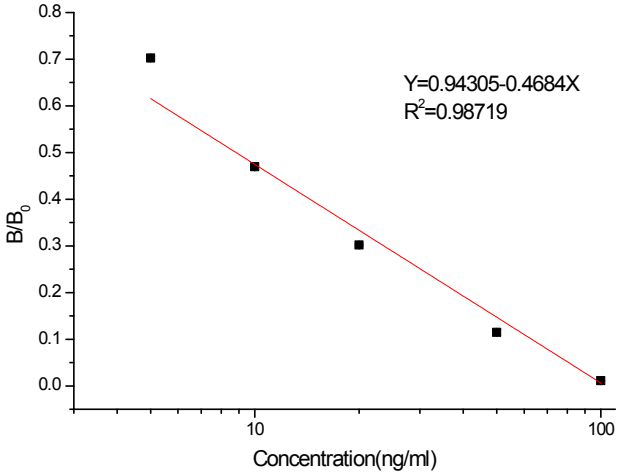


Pbs Serum

(B) amoxicillin

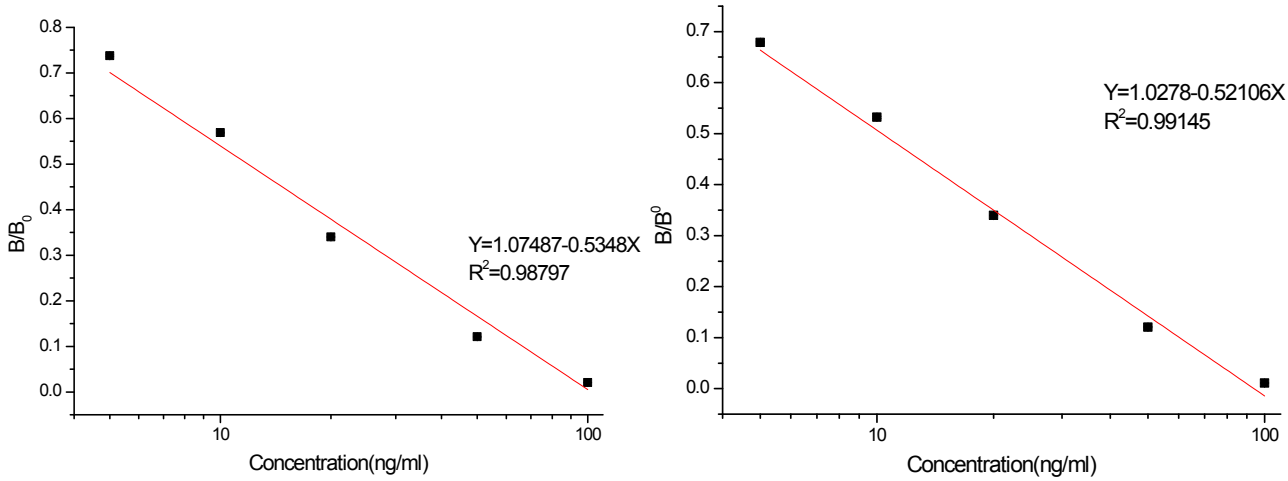


Pbs



Serum

(C) cefmetazole



Pbs Serum

Figure S4 Workflow of LFRA

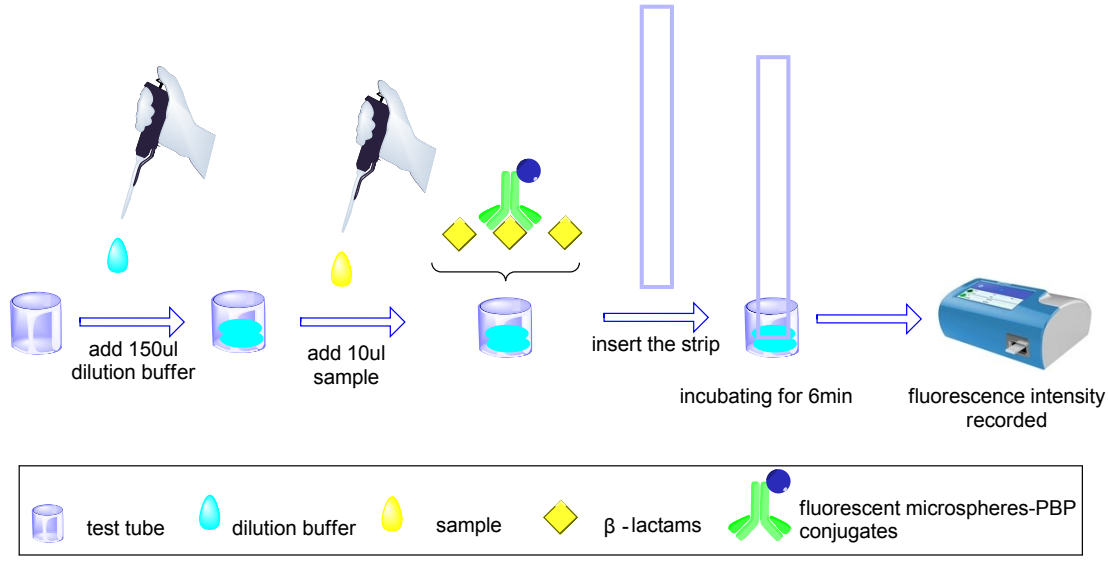


Table S1 The comparison of the proposed method with reported studies

Method	System	Detection limit (ng/mL)	Compound quantity	Time	Reference
Lateral flow fluorescent assay	Europium chelate-labeled lateral flow assay for rapid and multiple detection of β-lactam antibiotics by the penicillin-binding protein	4-500	25	10 min	This work
Electrochemical assay	Anti-penicillin G conjugation was used to develop a competitive immunosensor assay for the detection of penicillin G and other β-lactam antibiotics	5	1	60 min	1
	Electrochemical technique was used for the elaboration and characterization platform of ampicillin based on surface plasmon resonance	430	1	50 min	2
	Electrochemical aptasensor for ampicillin detection based on the protective e□ect of aptamer-antibiotic conjugate towards DpnII and Exo III digestion	0.013	1	80 min	3
	Immobilized enzyme penicillinase (Pen X) was modified onto the modified electrode to prepare a biosensor	0.64	1	40min	4
Colorimetric assay	Ninhydrin, p-dimethylaminobenzaldehyde, and Fehling's reagent are used as reagents to develop paper-based test for amoxicillin	$1.5*10^6$	1	10 min	5
	Colorimetric assay for ampicillin was developed based on Cu-BCA complexation	$26*10^3$	1	30 min	6
chemiluminescence assay	Chemiluminescence (CL) micro-flow system combined with on-line solid phase extraction (SPE) was used for determination of β-lactam antibiotics (penicillin, cefradine, cefadroxil, cefalexin) in milk	40-500	4	20 min	7
	Enhanced chemiluminescence of carminic acid permanganate by CdS quantum dot	5.8	1	30 min	8
Mass spectrometry	Ultra-high-performance liquid chromatography-tandem mass spectrometry	500-1000	7	20 min	9
	Liquid chromatography coupled with tandem mass spectrometric	1000-2000	8	15 min	10

References

[1] G. Merola, E. Martini, M. Tomassetti, *J. Pharmaceut. Biomed*., 2015, **106**, 186.

[2] A. Blidar, B. Feier, M. Tertis, *Anal. Bioanal. Chem*.,2019, **411**, 1053.

[3] T. Wang, H. Yin, Y. Zhang, *Talanta*, 2019, **197**, 42.

[4] Y. Xiu, R. Luo, B. Han, Food Anal. Method., 2020, **13**, 617.

[5] A.V. Marakaeva, I.V. Kosyreva, *Chem. Pap*., 2020, **74**, 2381.

[6] S. Kaur, M. Garg, S. Mittal, *Sensor. Actuat. B-chem*., 2017, **248**, 234.

[7] W. Liu, Z.J. Zhang, Z.Q. Liu, *Anal. Chim. Acta*, 2007, **592**, 187.

[8] A. Khataee, A. Hasanzadeh, R. Lotfi, *Talanta*, 2016, **152**, 171.

[9] R.R. Bonnin, A. Ribera, A.A. Roca, *Anal. Chim. Acta*, 2017, **468**, 215.

[10] R. Bellouard, G. Deslandes, C. Morival, *J. Pharmaceut. Biomed*., 2020, **178**, 112904.