Electronic Supplementary Material (ESI) for Analytical Methods.

A simple and rapid strategy for monitoring of vancomycin concentration in serum using a multicolor immunosensor based on the ratio of gold nanobipyramids and a product of cetyltrimethylammonium bromide-blue oxide of 3, 3′, 5, 5′-tetramethylbenzidine interaction

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S1 VAN detection of serum samples using LC–MS/MS

The LC-MS/MS method was based on several previous reports with minor adjustments, using methotrexate as an internal standard (IS) [1]. The chromatographic separation was performed on an ACQUITY UPLC system with a HSS T3 column (150 mm × 2.1 mm, 1.8 μm) (Waters, USA). The Chromatographic conditions are as follows: column temperature, 40 ℃; injector temperature, 10 ℃; injection volume, 2 μL; mobile phase A, 0.1% (volume) formic acid in acetonitrile; mobile phase B, 0.1% (volume) aqueous formic acid; flow rate, 0.3 mL/min; gradient elution procedure, 0-1 min, 5% A; 1-2 min, 30% A; 2-2.5 min, 5% A; 2.5-4.5 min, 5% A. A TQS mass spectrometer was coupled to the UPLC system with an electrospray ionization (ESI) interface and operated in multiple reaction monitoring (MRM) mode. The detailed parameters for the mass spectrometer are were as follows: capillary voltage, 3 kV; source offset, 50 V; desolvation temperature, 550 °C; source temperature, 150 °C, desolvation gas flow, 800 L/h; cone gas flow, 150 L/h; nebulizer gas, 7.0 bar; collision gas, 0.13 mL/min. The specific data of precursor/product ions were 725.36 > 144.16 and 725.36 > 100.14 for vancomycin, and 455.203 > 308.15 and 455.203 > 175.11 for methotrexate as the internal standard. All data were evaluated using MassLynx 4.1. software (Waters, USA). LC-MS/MS Method Validation The specificity, linearity, precision and accuracy data of LC-MS/MS method was shown in Fig.S9, Fig.S10 and Table S1.

S2 Acquisition of RGB values in photos

In this work, we obtained 11 RGB values from each photo of representative multicolor solution (purple, blue and pink) and calculate the values of R/B for each

color area. As shown in Fig.S11, R/B values of solution in the bottom area is similar. Compare with bottom area, the R/B values near the microporous wall are lower (purple and pink) or higher (blue). In addition, the R/B values at the bottom area with white reflective spots are increased or decreased. Therefore, in order to minimize the impact of light during the photography process, we fixed obtain the RGB values in the bottom area of microcell and avoid white reflective dots to represent the color of the solution.

Reference

[1] Y Fan, X Peng, H Wu, X Liang, Y Chen, B Guo, J Zhang. Simultaneous separation and determination of vancomycin and its crystalline degradation products in human serum by ultra high performance liquid chromatography tandem mass spectrometry method and its application in therapeutic drug monitoring. J Sep Sci. 43 (21) (2020) 3987-3994.

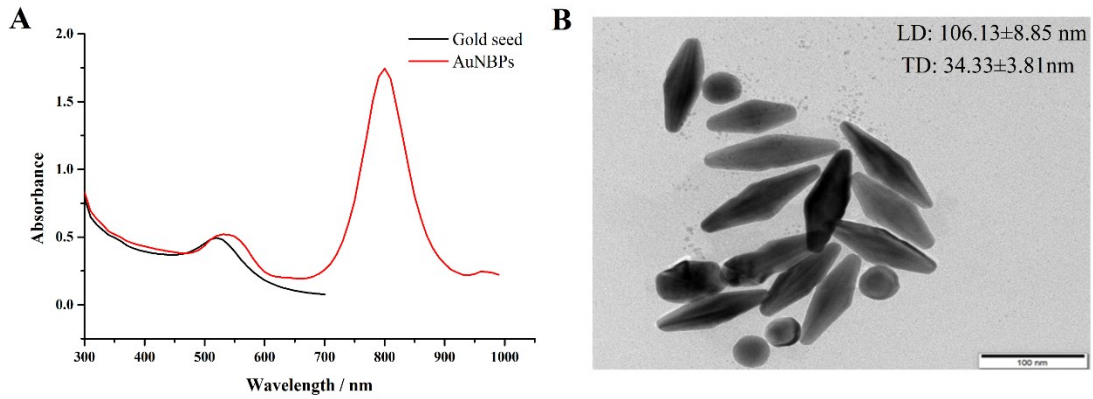

Fig. S1. UV−vis spectra of gold seed and AuNBPs (A); TEM images of AuNBPs (B).

LD: Longitudinal diameters (expression with mean ± standard deviation) ; TD: Transverse diameters (expression with me.an ± standard deviation).

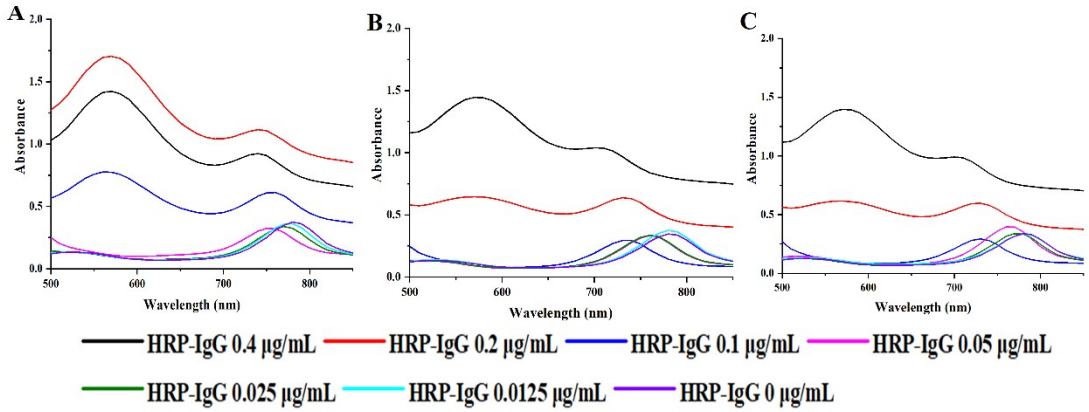

Fig. S2. UV−visible spectra of reaction system with 0.25 M HCl (A), 0.5 M HCl (B) and 1 M HCl (C)

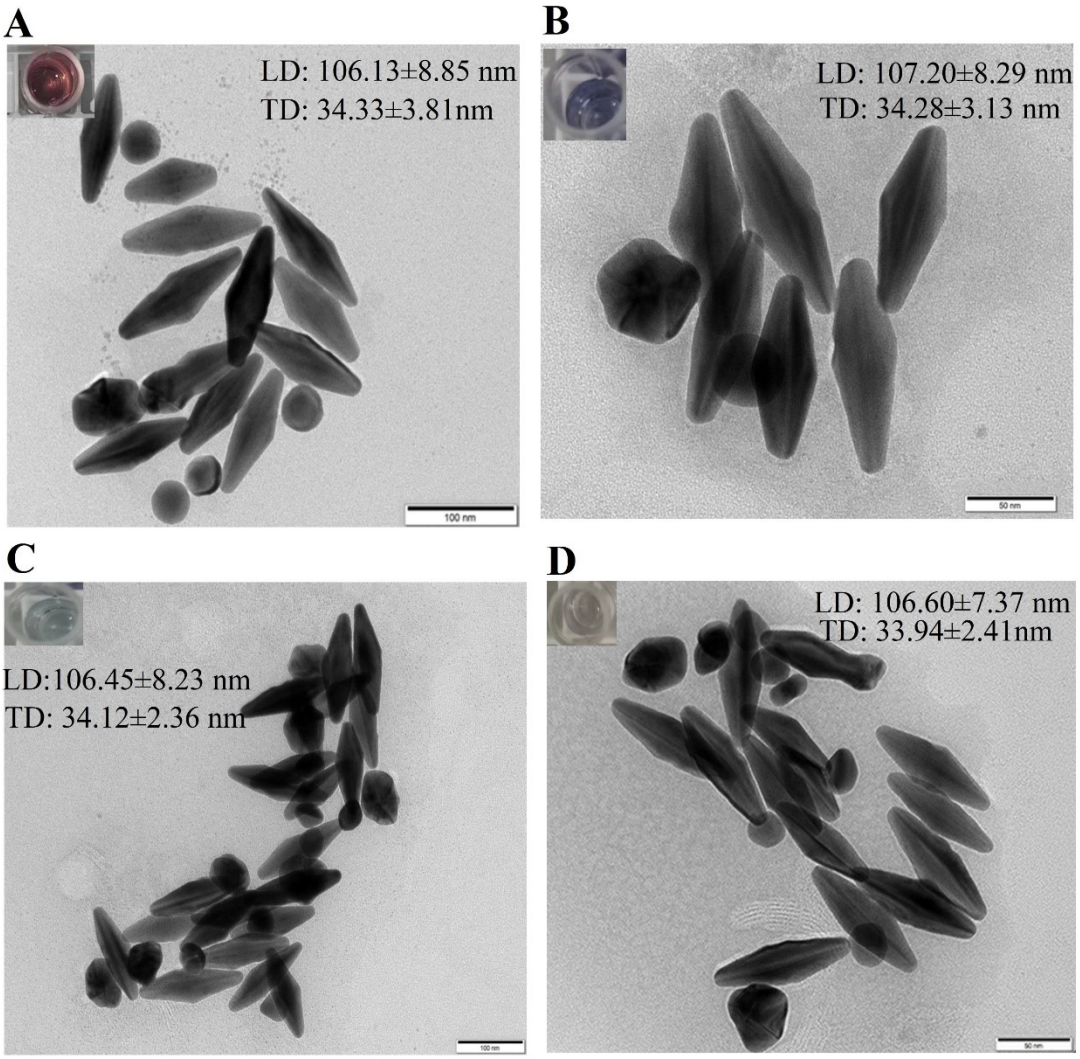

Fig. S3. TEM images of the AuNBPs with color change in detecting different VAN concentrations: The original AuNBPs (A); 2.5 μg/mL VAN (B); 10 μg/mL VAN (C); 20 μg/mL VAN (D).

LD: Longitudinal diameters (expression with mean ± standard deviation); TD: Transverse diameters (expression with me.an ± standard deviation).

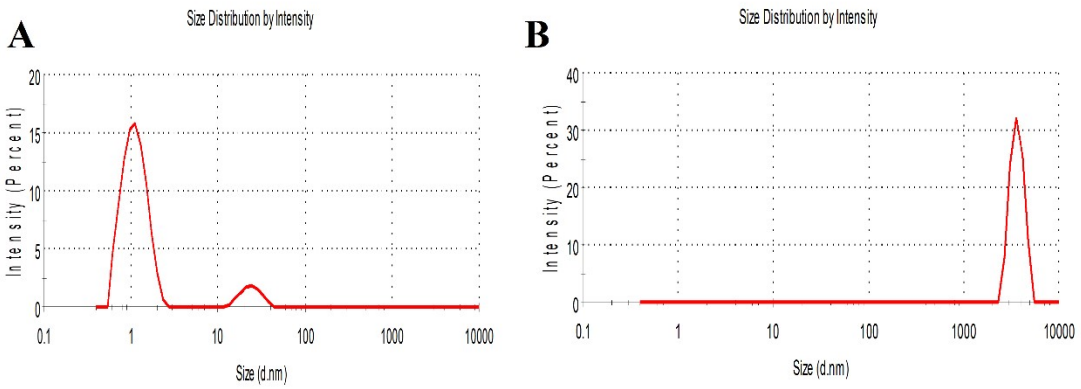

Fig. S4. TEM images of the AuNBPs with color change in detecting different VAN concentrations: The original AuNBPs (A); 2.5 μg/mL VAN (B); 10 μg/mL VAN (C); 20 μg/mL VAN (D).

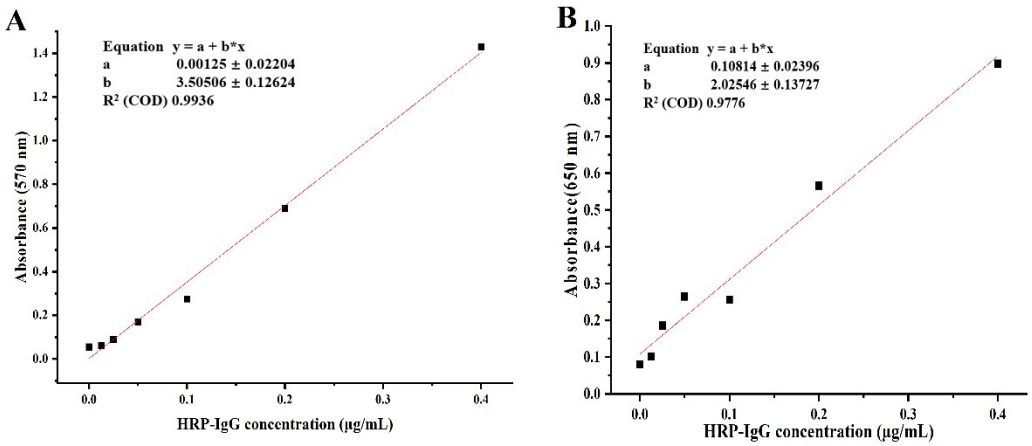

Fig. S5. The correlation curve for HRP-IgG concentration and absorbance

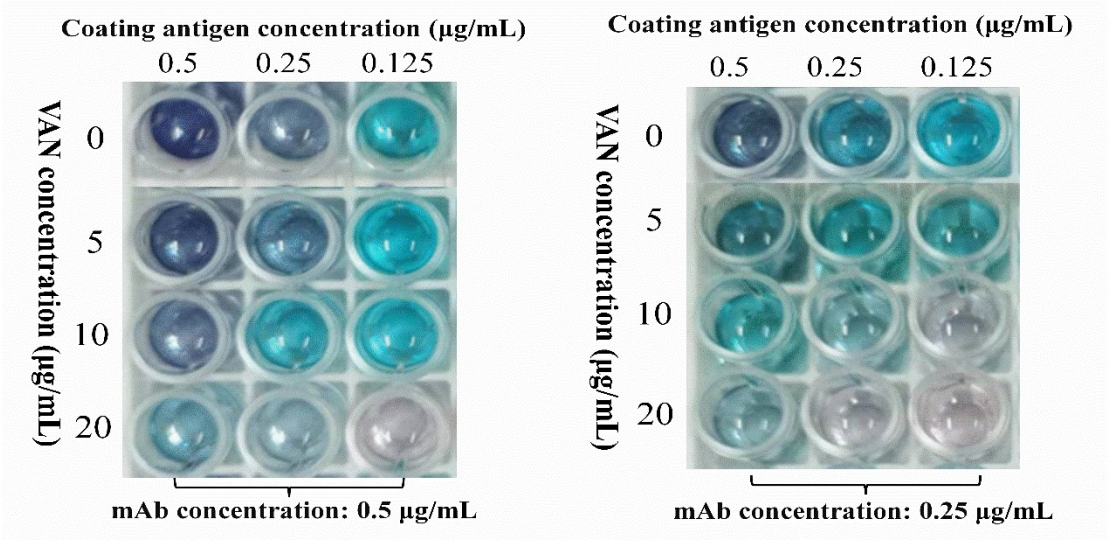

Fig. S6. Scanned images of the TMB/AuNBPs solutions with different level of coating antigen and mAb

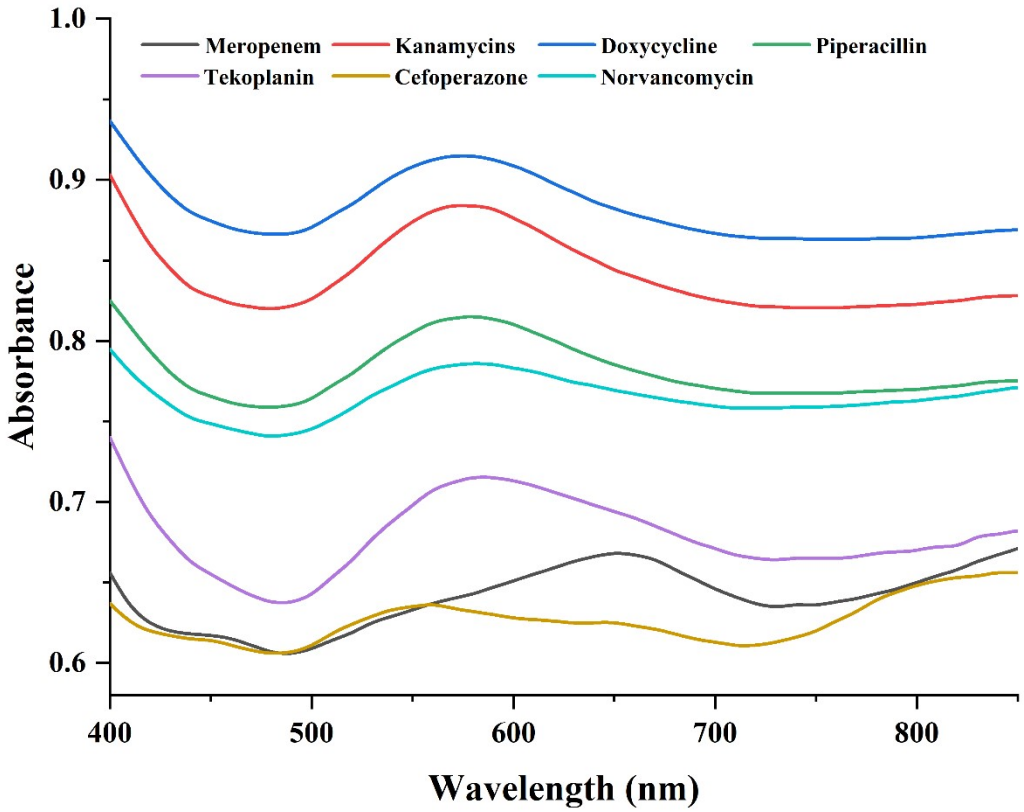

Fig.S7 The UV–vis spectra of different targets for cross-reactivity

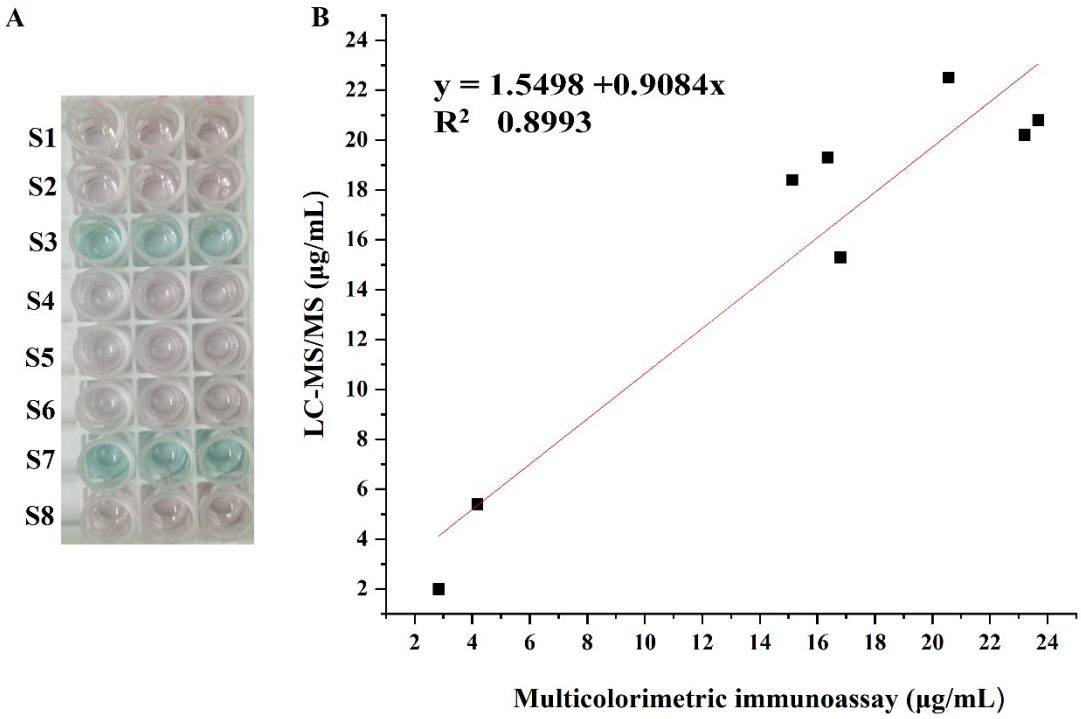

Fig. S8. (A) Scanned image of the TMB/AuNBPs solution with 8 batches serum samples (n=3) and (B) correlation curve for VAN in serum analyzed using multicolor immunosensor and LC-MS/MS.

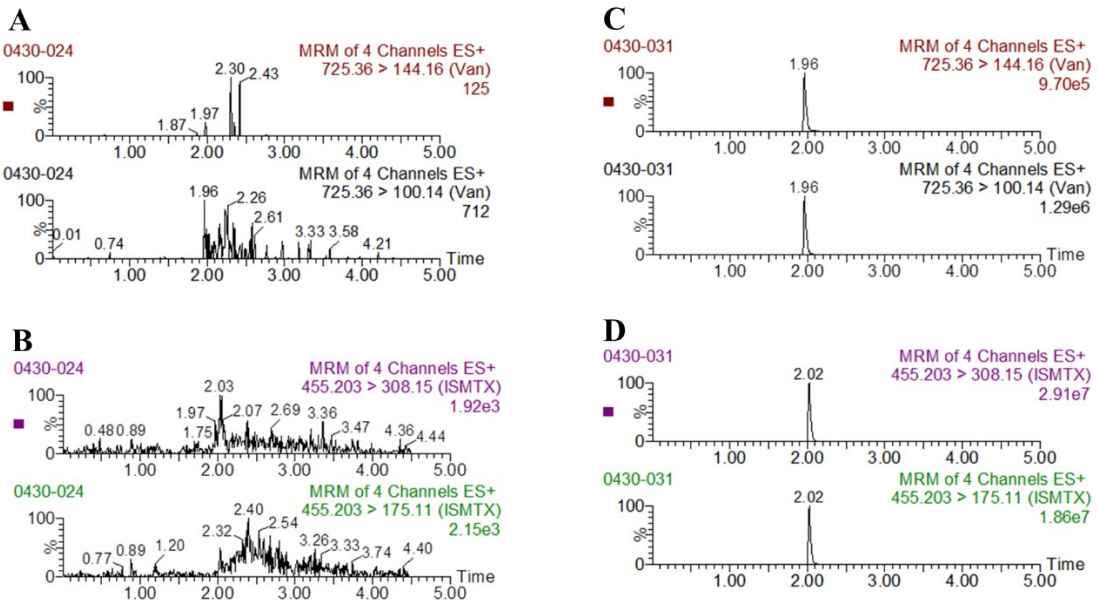

Fig.S9. Representative LC–MS/MS chromatograms containing (A) and (B) Blank serum sample, (C) Blank serum spiked with VAN, (D) Blank serum spiked with IS (MTX).

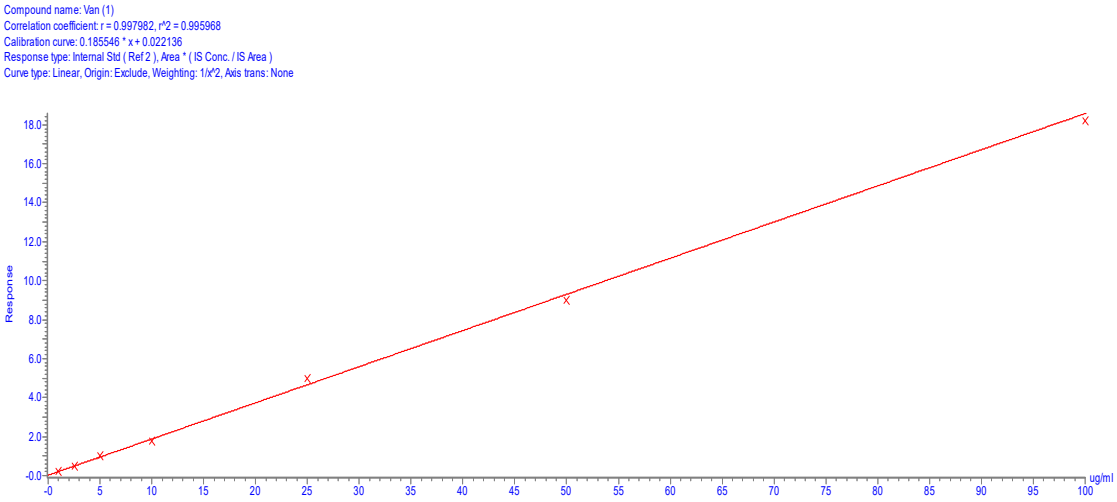

Fig.S10. The standard curves and linearity for LC-MS/MS analysis of VAN.

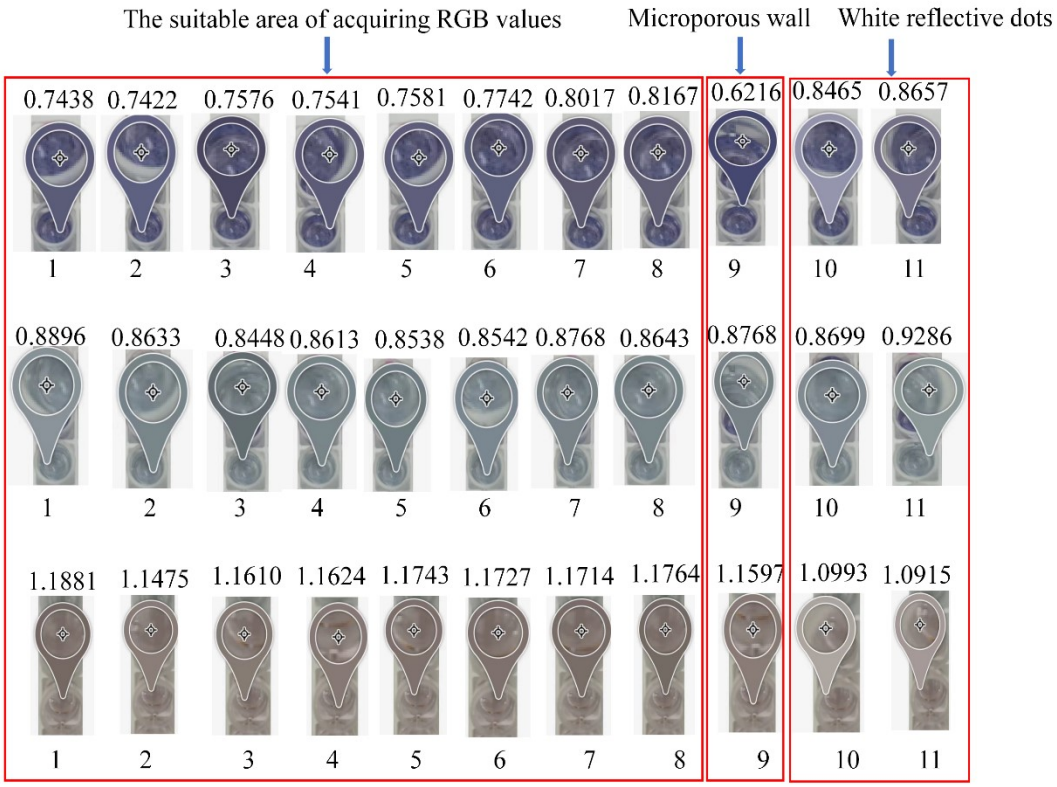

Fig.S11. R/B values from representative photos of multicolor solutions

Table S1 Accuracy and precision for vancomycin in human serum of LC-MS/MS Assay. (n=5)

Concentration (μg/mL)	Intrabatch		Interbatch	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
2	106.0%	6.29	98.79	13.8
8	97.3	4.05	94.9	3.81
80	94.5	3.81	105.6	6.7