The development of lateral flow immunoassay for monitoring vancomycin in peritoneal dialysate

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Purpose

We aim to develop a method to measure vancomycin (VAN) concentration in a peritoneal dialysate within 20 min by applying the existing paper-based mixing technology to mix and flow the sample solution and the PBST assay buffer directly on paper.

Introduction

Peritonitis is a major cause of peritoneal dialysis (PD) failure, necessitating appropriate antibiotic treatment. VAN is used empirically to treat peritonitis, but its potential nephrotoxicity at high doses requires careful monitoring of its concentrations. [1, 2]. Generally, VAN concentration monitoring is performed in serum, but monitoring VAN concentrations in dialysate may be more appropriate for PD patients who receive intraperitoneal administration [3]. This method also allows for easier and more accessible monitoring, as patients can collect samples themselves.

Working principle

Competitive lateral flow immunoassay (LFIA) is based on the competitive binding reaction between the target in the sample and the target (competitor) immobilized on the NC membrane. Without the antigen, the VAN antibody binds to VAN-BSA on the test line, forming a red line, while unbound AuNPs bind to the control line. Conversely, the VAN antibody binds to VAN in the sample, resulting in no red line on the test line (Figure 1). In the mixing experiment, the principle involves using constricted-expanded structures to enhance fluid dynamics [4].

Sample preparation

For a selectivity test, each antibiotic was spiked into PD solution at a concentration of 100 μg/mL. For the detection range test, a 10 mg/mL VAN solution was diluted with PD solution to create samples with various VAN concentrations. blue and yellow inks were used for the mixing experiment.

Experiment procedure

Samples were diluted 1:9 with assay buffer and analyzed using the dipstick method with a 20 min assay time. Results were obtained via the ChemiDoc XPS+ system, and line intensity values were measured with Image Lab software. For the mixing experiment, blue and yellow inks were injected through a microfluidic device onto paper at the outlet of the device.

Results

Figure 2 shows the cross-reactivity test results of the LFIA strip with several antibiotics to evaluate its selectivity. Most antibiotics showed intensity values similar to negative samples, while VAN showed lower intensity values compared to negative samples. Detection experiments with varying VAN concentrations (0 − 100,000 ng/mL) showed decreased test line intensity with increasing concentration (Figure 3). Logistic fitting produced a calibration curve with an R^2 of 0.987. Figure 4 shows the experimental results of mixing efficiency according to channel width. The color distribution was quantified from the obtained images, with higher distributions resulting in higher standard deviation (STD) values. The 0.5 mm condition showed the smallest STD value, indicating the highest mixing efficiency.

Conclusion

The developed LFIA strip demonstrated high selectivity and sensitivity for VAN detection in PD solution. A strong correlation ($R^2 = 0.98$) was observed between VAN concentration and test line intensity. Paper-based mixing experiments indicated that the 0.5 mm channel width had the highest efficiency. Future work should combine the mixing paper with the LFIA strip and validate with actual PD patient samples.

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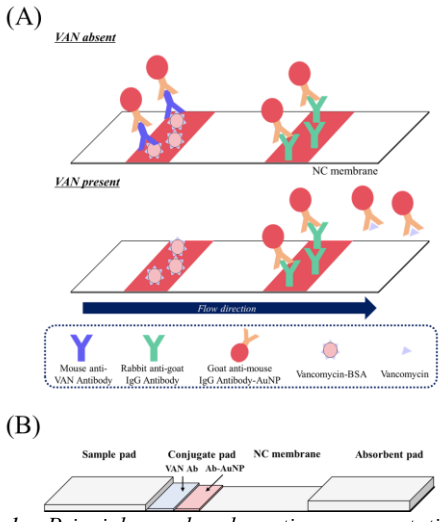

Figure 1: Principle and schematic representation of the competitive LFIA strip for VAN detection. (A) Mechanism of the LFIA strip. (B) Schematic of the LFIA strip components.

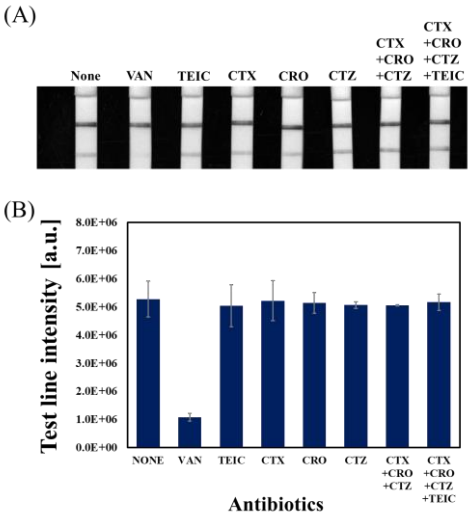

Figure 3: Selectivity test of the LFIA strip with various antibiotics. (A) strip images for different antibiotics: None (control), Vancomycin (VAN), Teicoplanin (TEIC), Cefotaxime (CTX), Ceftriaxone (CRO), Ceftazidime (CTZ), and combinations. (B) Test line intensity values for each antibiotic (N=3).

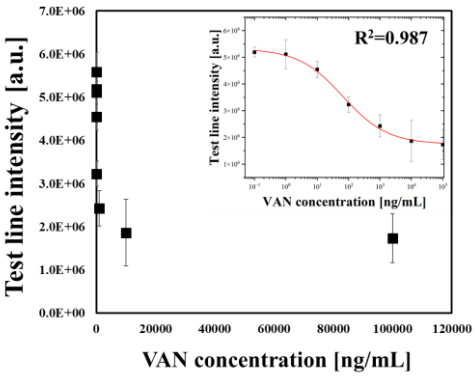

Figure 4: Detection range of the LFIA strip for VAN. The graph shows the test line intensity at different VAN concentrations. The inset displays the calibration curve with an R^2 value of 0.987, indicating a strong correlation between VAN concentration and test line intensity (N=3).

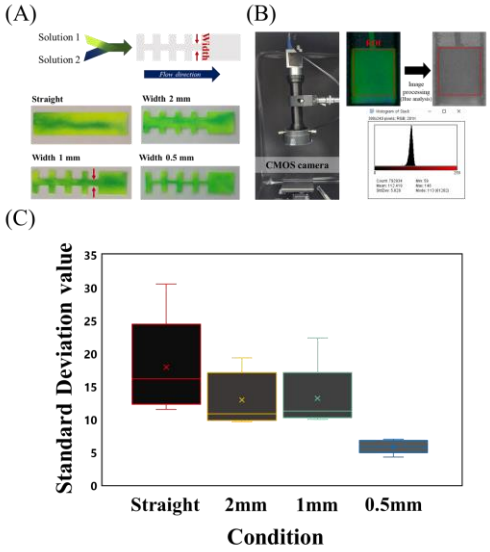

Figure 4: Evaluation of mixing efficiency according to channel width. (A) mixing experiment and result image. (B) Analysis method (C) box plots of standard deviation values for each channel width (N=5).

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