ELISA-based Detection of Gentamicin and Vancomycin

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Acknowledgments:

Disclosures:

Methods: A gentamicin- or vancomycin-protein conjugate was established by cross-linking either gentamicin or vancomycin to bovine albumin using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). The non-crosslinked antibiotic fraction was removed from the conjugate by several repetitive dialysis steps. Purified conjugate coated overnight to the surface of a microtiterplate (10 ng gentamycin-BSA or 1 µg vancomycin-BSA) in a 50mM bicarbonate buffer (pH 9.6). After washing the plate four times with PBS containing 0.05% Tween-20, the microtiterplate wells were treated, for 1 hour, with PBS containing 5% BSA as an additional blocking step. The calibration curve for both antibiotics was prepared in PBS containing 5% BSA with a range of 0.1 ng/ml - 500 ng/ml for either gentamicin or vancomycin. The 1 hour incubation period of the calibration curve in the microtiterplate was combined with the incubation of the antibiotic specific primary antibody (the monoclonal anti-gentamicin antibody was diluted 7000x in PBS/BSA, the polyclonal anti-vancomycin antibody was diluted 5000x) in the same well. After incubation the wells were washed as described above, after removal of the washing liquid the wells were incubated, for 1 hour, with the secondary antibody (for gentamicin: RAMPO, 5000x diluted in PBS/BSA, for vancomycin: SWARPO, 2000x). Subsequently the wells were washed as described above. After washing, the antibody fraction attached to the coated surface was detected by the use of an HRP-conjugated secondary antibody and subsequent conversion of a tetramethylbenzidine (TMB) substrate (measured at 450 nm in an ELISA plate reader). The intensity of the 450 nm signal is inversely correlated to the concentration of the antibiotic in a sample.

Results: BSA-gentamicin and BSA-vancomycin haptens were generated to immobilize both antibiotics to the microtiterplate wells. Using fixed concentrations of the anti-gentamicin or anti-vancomycin antibodies in combination with increasing concentrations of free antibiotic as a calibration series (range: 0.1 ng/ml - 500 ng/ml), lead to an A450 signal that was dose dependently inversely correlated to the antibiotic concentration. The results were calculated in a log/log fashion, and the detection range was determined by polynomial regression. The established calibration curve allowed a detectable range between 2 - 300 ng/ml for gentamicin (Figure 1A) and 10 - 500 ng/ml for vancomycin (Figure 1B). To determine whether this ELISA setup supports the detection of gentamicin and vancomycin in high-protein containing samples (wound exudate and human serum), we spiked wound exudate with 5 µg/ml gentamicin and human serum with 50 µg/ml vancomycin. After sample dilution we were able to measure 4.5 ng/ml gentamicin in the diluted wound exudate and 53.5 ng/ml vancomycin in the diluted human serum, corresponding to 4.5 µg/ml gentamicin and 53.5 µg/ml vancomycin in the crude samples. Importantly, no cross-reactivity or interference was observed for vancomycin in the gentamicin ELISA and vice versa.

Discussion: Our results show that ELISA provides in a highly sensitive method to measure antibiotic levels in both wound exudate and serum. In contrast to the in literature described fluorescent detection methods, the herein described ELISAs are about 50x more sensitive. Since our ELISA-based method is compatible with high-protein containing samples, measurements in wound exudate and serum are no longer a practical obstacle as they are for liquid chromatographic methods. The application of these ELISAs may contribute to an improved antibiotic regimen in the clinic for osteomyelitis treatment.

Significance: Gentamicin and vancomycin concentrations in various types of samples can be determined by the use of commercially available antibodies in a hapten-supported ELISA-based approach. Both ELISAs are highly sensitive and for the individual antibiotics and in both ELISAs no cross-reactivity was found for other antibiotics.

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Figure 1A: Calibration curve of the gentamicin ELISA. B: Calibration curve of the vancomycin ELISA. Dashed lines indicate the detectable range of the assay. Note that the A450 signal is reversely correlated with the concentration of the respective antibiotics.

ORS 2014 Annual Meeting
Poster No: 1948