**Development of a antibiotics screening immunoassay and its validation in raw milk**

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Antibiotics are a diverse group of antibacterial compounds and are used to treat bacterial infections in humans and animals. The intensive use of antibiotics in animal husbandry and public health care has resulted in an increase in the strains and numbers of antibiotic resistant bacteria. Therefore, the use of antimicrobials was restricted by the European Union in 2006. Despite this restriction, the presence of antimicrobial residues and the dissemination of antimicrobial resistance is still regarded as an emerging problem. Monitoring food from animal origin for the presence of antimicrobials is obligatory and extensive monitoring programs are in place within the European Union. While confirmatory analysis is mostly LC-MS/MS based, screening methods used are very diverse. Since antibiotics are comprised of different structural compounds, a multiplex screening approach is favourable. Our aim was to develop a multiplex immunoassay based screening assay that detects relevant antibiotics. To this end we chose the Luminex xMAP technology and the MAGPIX system. The combination of the xMAP technology and the detection system, were important for our main goals. Besides multiplexing, the assay should be fast, easy to use, transportable and applicable on-site and at-point. The developed competition-based assay consists of antibiotic-protein conjugates coupled to beads and monoclonal antibodies raised against the target antibiotics. The developed multiplex detects single antibiotics as well antibiotic groups like aminoglycosides, sulfonamides, tetracyclines, (fluoro)quinolones, β-lactams and lincosamides. The antibiotics multiplex was validated as a screening assay for raw milk and drinking water (from calf and pig farms) and the suitability for on-site and at-point testing was evaluated. The sensitivities for all antibiotics in animal drinking water and raw milk were well below 100 ng/mL and in most cases even below 10 ng/mL. Currently the method is further developed to include more antibiotic targets as well as other contaminants in raw milk. This screening assay can support governmental inspections and might help to further reduce the (ab)use of antimicrobial in the animal production. Because of its low sensitivities it may also applicable for monitoring environmental water samples. The emission of antibiotics into the environment should be reduced as an important part of the risk management.